IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Geoffrey N. Roth and Jonathan N. Roth

Serial No:

Filed: January 7, 2002

Art Unit: 1631

For: TEST MEDIA AND QUANTITATIVE OR QUALITATIVE METHOD FOR IDENTIFICATION AND DIFFERENTIATION OF BIOLOGICAL MATERIALS IN A TEST SAMPLE

Commissioner for Patents and Trademarks
Washington DC 20231

Dear Sir or Madam:

PRELIMINARY AMENDMENT

Before examination on the merits, please amend the Application as follows:

In the Claims

For this Continuation Application, please cancel all previous claims.

Please add new claims as follows:

72. A test medium for detecting, quantifying and differentiating general coliforms, *E. coli,* and at least one of the genera *Aeromonas and Salmonella,* said test medium comprising: a nutrient base medium including ions of a salt; a first substrate which forms a first component of a first color in the presence of *E. coli;* a second substrate which forms a second component of a second color in the presence of general coliforms; and a third substrate which forms a third component of a third color in the presence of one of the genera *Aeromonas* and *Salmonella,* all of said colors being distinguishable from one another; said first substrate being a β-glucuronide nonchromogenic substrate; said second and third substrates being chromogenic substrates; and said first color being substantially black.

73. The test medium of claim 72, wherein said first substrate is selected from the group consisting of 8-hydroxyquinoline-β-D-glucuronide, an esculetin glucuronide, and cyclohexenoesculetin-β-D-glucuronide.

74. The test medium of claim 72, wherein said third substrate forms said third component of said third color in the presence of one of *Salmonella*, and said medium includes an inhibitor for inhibiting colonies of *Aeromonas* and other *nonenterobacteriaceae spp.*

75. The test medium of claim 72, wherein said second substrate comprises an α-galactoside and said third substrate comprises a β-galactoside.

76. The test medium of claim 72, wherein said first substrate is 8-hydroxyquinoline-β-D-glucuronide and forms a substantially nondiffusible compound in the presence of ions of said salt and *E. coli* and said third substrate also forms said third component of said third color in the presence *Shigella*.

77. The test medium of claim 72, wherein said third substrate also forms said third component of said third color in the presence of general coliforms, and wherein said second and said third colors are combined to form components of a fourth color in the presence of general coliforms, said fourth color being distinguishable from said first, second, and third colors.

78. The test medium of claim 77, wherein said third color forms in the presence of *Aeromonas*, and said second substrate also forms said second component of said second color in the presence of *Salmonella.*

79. The test medium of claim 72, wherein said salt comprises a metallic salt and said first component is water insoluble as formed by reaction with said ions.

80. The test medium of claim 75, wherein said first substrate consists essentially of 8-hydroxyquinoline- β-D-glucuronide, said second substrate consists essentially of 5-bromo-4-chloro-3-indole-α-D-galactoside, and said third substrate consists essentially of 6-chloro-3-indole-β-D-galactoside.

81. A method for detecting, quantifying, and differentiating colonies of *Aeromonas* from selected other biological entities in a test sample, said method comprising the following steps: providing a base medium including ions of salt, a β-D-galactoside substrate that forms a first component of a first color in the presence of a first enzyme, an α-D-galactoside substrate that forms a second component of a second color distinguishable from said first color in the presence of a second enzyme, and a β-glucuronide nonchromogenic substrate that forms a third substantially black component in the presence of a third enzyme; inoculating the test medium with a test sample; incubating the test medium; and examining the test medium whereby aggregations of colonies of *Aeromonas* are indicated by said first color, and aggregations of colonies of *Salmonella* are indicated by said second color, and whereby colonies of general coliforms are indicated by a third color, said third color being a combination of said first and second colors.

82. The method as set forth in claim 81, further comprising the step of examining the test medium for *E. coli* as indicated by the presence of substantially black aggregates.

83. The method as set forth in Claim 82, wherein said β-glucuronide substrate is 8-hydroxyquinoline and further comprising the step of examining the test medium for *Shigella*, which are also indicated by said second color.

REMARKS

In response to the Office Action of July 26, 2001 on parent application (Serial No. 09/357,606), Applicant cancelled all of the then pending claims except claims 66-68, which an agreement of allowability without the need for further search had been reached with the Examiner. This Continuation Application is brought to pursue subject matter in those cancelled claims, which Applicant considers to contain patentable subject matter, and is not intended to interfere with the issuance of the patent on parent application 09/357,606. Accordingly, the above claims 72-83 correspond to previously cancelled claims 31-37, 39, and 69-71 respectively of the parent application.

In addition to the following remarks, Applicant reaffirms the comments in the responses filed on August 2, 2000, February 2, 2001, and June 28, 2001 in the parent application as if fully rewritten herein. In the Office Action of July 26, 2001, the main contention of the Examiner is that Applicant did not provide convincing evidence that *Aeromonas* will not react with the β-glucuronide substrate. Furthermore, the Examiner asserted that Sartory teaches that about thirty percent (30%) of *Aeromonas* were glucuronidase positive when tested in this assay (page 1665). (Exhibit A). Applicant respectfully disagrees with this contention as Sartory's study ignores any *Aeromonas* that did not test positive for either lactose or glucuronide. The purpose of Sartory's test was to identify coliforms and *E. coli* utilizing a m-LGA medium; and therefore, he was not concerned about *Aeromonas* that provided no positive results for lactose or glucuronidase in this medium. The Examiner's assumption also ignores that it is likely the medium contained other $lac^{+}gluc^{-}$ strains of a *Aeromonas* in addition to the identified 36 strains of *Aeromonas hydrophila/cavie*.

Aeromonas was identified in the Sartory medium by testing colonies that had a positive reaction to the m-LGA medium for oxidase reaction. (page 1664, 2nd paragraph) Oxidase reaction is one of the preeminent characteristics of *Aeromonas*; however, there is no indication that Sartory tested for oxidase reaction for any *Aeromonas* colonies unless they already had a positive reaction to either the m-LGA or m-LSB mediums. Furthermore, in the attached article from Sartory, D. and Howard, L., *A medium detecting β-glucuronidase for the simultaneous membrane filtration enumeration of Escherichia coli and coliforms from drinking water,* Letters in Applied Microbiology, 1992, 15, 273-276 (Exhibit B), which documented the study that Sartory's presentation at the 1992 Water Quality Technology Conference was based upon, Sartory notes that "representative colonies from m-LSB and m-LGA were picked off for tube confirmation and oxidase reaction..." (page 274, lines 28-31). Also, in Table I of this article, it can be seen that 133 of the 761 yellow colonies found in m-LGA medium tested positive for oxidase. It is likely that most of these are *Aeromonas*. Therefore, it is also likely that there were many more colonies of *Aeromonas* that tested lac^{+}

gluc$^-$ in the medium than the 36 colonies specifically identified as *Aeromonas hydrophila/caviae*. Accordingly, it cannot be concluded that thirty percent (30%) of *Aeromonas* were glucuronidase positive as it ignores any *Aeromonas* present that were not of the species *hydrophila/caviae* or that did not test positive for either lactose or glucuronidase.[1] Furthermore, Sartory makes no attempt to dispute the reported findings of Kaznowski that only 3.1 percent of strains of A. hydrophila, 4.1 percent of A. sobria and none of A. caviae were glucuronidase-positive.

Applicant, Dr. Jonathan N. Roth, has also executed a Declaration (attached Rule 132 Declaration of Dr. Jonathan N. Roth) that includes an Attachment of an e-mail Dr. Roth sent to Dr. David Sartory inquiring about the implications of his study, and a reply from Dr. Sartory. Dr. Sartory acknowledges that based on his experience the vast majority of glucuronidase-negative, oxidase-positive yellow colony isolates on m-LGA would be species of *Aeromonas*. Dr. Sartory also states that on a separate, unpublished study he undertook specifically on *Aeromonas,* only 1 isolate of 203 from drinking water was glucuronidase-positive and only 2.7% of *Aeromonas* isolates from river water were glucuronidase-positive in accord with the data of Kaznowski et al.

This also comports with the vast majority of other studies that indicate that *Aeromonas* species are glucuronidase negative. For example, please see the attached references as follows:

> Kilian, M., and P. Bulow, *Rapid Diagnosis of Enterobacteriaceae*. Acta Path. Microbiol. Scand. 1976, Sect. B, (84): 245-251. (Exhibit C).
> This study tested 633 strains of Enterobacteriaceae and Vibionaceae using chromogenic substrates, including 113 *E. coli* and 18 *Aeromonas spp.* 97% of the *E. coli* were glucuronidase positive and none of the *Aeromonas spp.* was glucuronidase positive. (p. 249, 2nd column and 250, 2nd column, 2nd paragraph)
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> Waltman, WD 2nd et al., *Enzymatic Characterization of Aeromonas hydrophila Complex by the API ZYM System.* J. Clin. Microbiol. 1982 Oct;16(4):692-6 (Exhibit D).
> This study states that 47 of 48 isolates tested negative for beta-glucuronidase. (p. 693, bottom 1st column and top 2nd column). Waltman also references A. Ljungh, M. Popoff, and T. Wadstrom, *Aeromonas hydrophila in acute diarrheal disease: detection of enterotoxin and biotyping of strains.* J. Clin. Microbiol. 1977, (6):96-100 (Exhibit E-part of Exhibit D) as also finding all

[1] It should also be noted that in Sartory's peer reviewed article in Applied Microbiology, there is no mention of the 22 blue colonies that were lac$^-$ gluc$^+$ that were discussed at the Water Quality Technology Conference.

11 isolates of *Aeromonas* hydrophila in their study to be negative for beta-glucuronidase. (Waltman, p. 695, 1st column, 2nd paragraph).

Janda, MJ, *Biochemical and exoenzymatic properties of Aeromonas species.* Diagn. Microbiol. Infect. Dis. 1985, (3):223-232. (Exhibit F).
This study reports that 92% of 64 isolates representing 3 different Aeromonas species were Beta-glucuronidase negative. (Table 6)

Austin, DA, D. McIntosh, and B. Austin, *Taxonomy of Fish Associated Aeromonas spp., with the Description of Aeromonas salmonicida subsp. smithia subsp.nov.* System. Appl. Microbiol. 1989, (11):277-290. (Exhibit G).
One Hundred eighty *Aeromonas* strains were compared and β-glucuronidase was sought using the API zym test kit. None of the *Aeromonas* was found to produce β-glucuronidase. (See highlighted text).

Kaznowski, A., Wlodarczak, K, & Paetz, H., *A Numerical Taxonomy of Vibrionaceae Isolated from Water, Sewage, Water-Oil Emulsion and Fishes.* System. and Appl. Microbiol. 1989, (12):172-178. (Exhibit H).
This study reported negative β-glucuronidase activity in 96.9% isolates of Aeromonas hydrophila, 95.9% isolates of Aeromonas sobria, and 100% isolates of Aeromonas caviae. (Table 1 and 2).

Aleksic, S., Bockemuhl, J., Schulze, G., Havemeister, G., Heinemeyer, E.A., Muller, H.E., von Pritzbuer, E., *Reactions of different Enterobacteriaceae and Vibrionaceae species in BRILA-MUG (Fluorocult) bouillion.* Zentralbl Hyg Umweltmed. 1990, (4):395-403. (Exhibit I).
This study included 16 Aeromonas strains which were isolated from surface water, none of which was beta-glucuronidase positive.

Kaznowski, A., *Identification of Aeromonas strains of different origin to the genomic species level.* J. Appl. Microbiol. 1998, (84):423-430. (Exhibit J).
This study states that 71 of 71 isolates tested negative for glucuronate as a sole carbon source. (page 425, 2nd column, last paragraph and 426, 1st column, 1st paragraph) This would mean that they must also be negative for beta-glucuronidase.

Accordingly, all claims are enabled and Applicant respectfully requests approval by Examiner of new claims 72-83 as added above. Should the Examiner have any questions or comments, she is invited to contact the below representative of the Applicant.

Respectfully submitted,

Daniel Tychonievich

Registration No. 41,358
Attorney for Applicant
BAKER & DANIELS
205 W Jefferson Blvd., Suite 250
South Bend IN 46601
(219)234-4149